PROJECT TITLE: FORENSIC ANALYSIS OF TRACE EXPLOSIVES

DR. DAVID S. HAGE, P.I.

UNIVERSITY OF NEBRASKA-LINCOLN

ABSTRACT

The rise in terrorist bombings has made the chemical identification of explosives a priority for the forensic scientist. The volume of evidence to be analyzed and the importance of such testing for preventative and investigative purposes have increased the need for detection methods specific for explosives that are reliable, fast, and cost effective. In this project, work was performed between the Chemistry Department at the University of Nebraska-Lincoln and the Nebraska State Patrol Crime Laboratory to develop new analytical methods for the forensic analysis of trace explosives by combining immunoextraction and capillary electrophoresis.

PROJECT DESCRIPTION

Currently, the state of Nebraska has no method for the chemical analysis of explosives. The analysis method created in this project for trace explosives makes use of antibody-based extraction (i.e., immunoextraction) for the concentration and selective isolation of desired explosive agents, followed by the separation and analysis of the isolated agents by capillary electrophoresis (CE). The immunoextraction sample pre-treatment and CE portions were developed separately and then merged to create a tandem analytical system.

High-performance liquid chromatography (HPLC) and gas chromatography (GC) are two common methods employed in analyzing explosives. CE is an alternative that has been explored for detecting explosives. Some of the advantages of CE versus HPLC include smaller sample requirements and greater efficiency, allowing CE to separate more compounds per run. CE, in contrast to GC, can work directly with liquid samples and its use of lower separation temperatures, which is important when dealing with thermally unstable explosive compounds. Both HPLC and GC are used for moderate to large amounts of sample. The CE method developed here utilizes nanoliter amounts of sample, unlike the milliliter sample amounts required in HPLC, and in a nondestructive manner (unlike GC). In addition, this CE method requires nanoliter amounts of other reagents.

PROJECT OBJECTIVES

An explosion crime scene presents unique challenges to the forensic scientist due to the volume of evidence that must be analyzed. A CE based detection method for explosives can aid in evidence analysis being reliable, fast, cost effective, and allowing for the non-destructive analysis of samples. The general objective of this study was to explore the use of antibody-based extraction with CE to meet these needs. The planned goals of this work were **1)** to develop small, reusable antibody columns for extraction of these agents from field samples, **2)** to combine this extraction with CE for separation and detection of explosives, and **3)** to evaluate this approach in the analysis of real forensic samples. Future work was then planned to examine the miniaturization of this system and development of a field-portable device.

PROCEDURES

The analysis method created in this project for trace explosives was based on immunoextraction for the concentration and selective isolation of desired explosive agents, followed separation and analysis of the isolated agents by CE. Immunoextraction is a superb choice for sample pre-treatment or clean-up and CE offers numerous benefits as a separation method. These two components are being developed separately to allow crime labs a choice in either sample pre-treatment or separation technique.

The antibodies to be used for immunoextraction were anti-TNT and anti-RDX preparations from Strategic BioSolutions (Newark, DE). The anti-TNT antibodies are known or bind many of the explosives that will be examined in this study [1], and were used to specifically isolate and concentrate these from samples. The anti-RDX antibodies were similarly used for other explosives or additives of interest. Trace analysis-grade samples of the explosive agents to be examined in this work were obtained from AccuStandard (New Haven, CT). Other materials were obtained from standard chemical suppliers such as Sigma/Aldrich, Fisher Scientific, and VWR.

The anti-TNT and anti-RDX immunoextraction columns were prepared according to current standard operating procedures (SOPs) in the PI's lab. The fabrication of these columns involved immobilizing the antibodies to diol-bonded silica through the Schiff base technique. The amount of immobilized antibodies was determined by a protein assay and their activity with approximately a dozen different explosives (including TNT and RDX) and explosive related agents was determined by frontal analysis.

Micellar electrokinetic chromatography (MEKC) was examined as the CE mode of analysis for two reasons: (1) use of MEKC for explosives analysis has been reported in

literature and (2) recent work in the PI's lab with herbicides highlighted the ability of MEKC to separate both charged and neutral agents and its ease of use with on- or off-line immunoextraction. Continuing work is being done to further optimize width and resolution of their individual peaks, their limits of detection, and the precision and total time of the analysis time (expected to be 5-10 minutes). Experiments have been conducted varying pH and ionic strength of the running buffer, the concentration and type of surfactant used for MEKC, the capillary length, and applied electric field in the pursuit of better separations. In addition, the amount of injected sample (generally in the nanoliter range) and the use of sample stacking methods have also been examined. Research in this area is on-going.

After optimizing both the immunoextraction and MEKC procedures, these are now being combined and evaluated for trace explosives detection. The immunoextraction was initially performed off-line followed by injection of the extracted agents into the CE system through the use of sample stacking (as used by the PI's lab for herbicide testing), but on-line coupling of immunoextraction with MEKC will also be investigated. Focus has been on the development of this techniques separately, but combination of this techniques will occur shortly.

This overall system was initially evaluated by using standard solutions of the desired trace explosives and/or additives. Items considered in this evaluation included limits of detection, analysis times, precision, overall response, and general sample requirements. Based on initial work with immunoextraction and CE, it is expected that the analysis time of the overall system will be approximately 15 minutes per sample.

Once tests have been completed with the overall system for standard samples, it will then be evaluated for use with field samples. This is being performed in collaboration with the Nebraska State Patrol Crime Lab. Following successful development of a laboratory-based system for explosives detection, future work will focus on the creation of a field-portable device that can be used for similar measurements. The PI's lab has conducted similar work in the development of a portable immunoextraction/HPLC system for environmental analysis. These systems are easily operated and transported by a single person being roughly the size of a small suitcase. These include a small portable power supply and laptop computer for data collection and system control. Similar devices will be useful for analyzing explosives at the site of a detonation or screening for the presence of a suspected explosive agent.

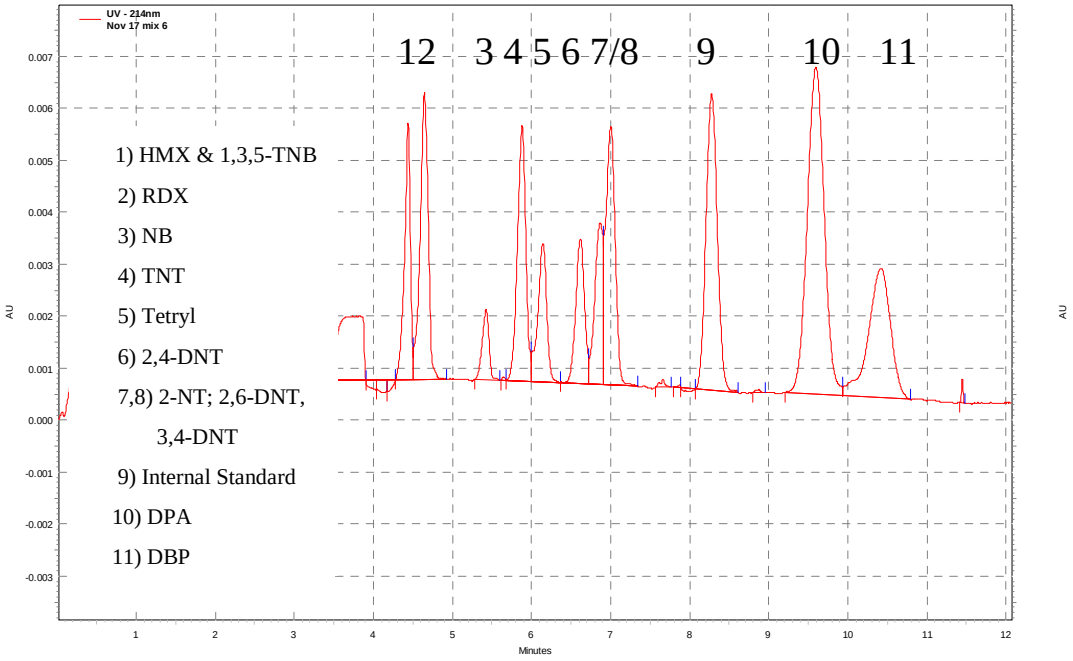
RESULTS/DISCUSSION

Approximately a dozen explosives including HMX, RDX, TNT, PETN, and Tetryl as well as explosives related organic molecules have been successfully separated using the CE method developed in this project. The current CE protocol has brought the following benefits:

- Nanoliter sample requirements, translating into lower consumption of evidence
- A total of 15 min analysis time per sample
- Smaller reagent requirements then currently employed methods lowers purchasing and waste disposal cost

Figure 1 shows an electropherogram obtained by this method for separation of a standard mix of fourteen explosives and explosives related compounds.

Figure 1. Separation & Analysis of Several Explosive Agents & Related Compounds by CE



The separation above was achieved utilized a mixed micelle (sodium dodecyl sulfate and 18-crown-6) MEKC borate buffer with a 40 cm effective length CE capillary utilizing single wavelength detection at 214 nm. New CE conditions are being explored to enhance the separation of the target organic compound for the purposes of complete resolution and shorter analysis times.

In addition, immunoextraction columns have been successfully developed with testing of these columns on-going. Based on past work that has been performed with similar columns for herbicides and pesticide analysis in environmental samples, the availability of such columns should greatly reduce the amount of time and effort that is required by forensic scientists to process explosives samples prior to analysis.

DISSEMINATION DISCUSSION

Dissemination of this research has been conducted through presentations made at the 2004 Joint Meeting of the Midwest, Mid-Atlantic, Southern, and Canadian Societies of Forensic Scientists. An invited presentation was also made on this work at a special meeting that was held in Fall 2004 by the U.S. Army Corps of Engineers on Biosensing Methods for Explosives. In addition, the PI's laboratory is planning to submit a paper on this topic for publication in the Journal of Forensic Science in 2005.